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THE FATE OF CUTANEOUSLY AND SUBCUTANEOUSLY IMPLANTED TRYPSIN PURIFIED DERMAL COLLAGEN IN THE PIG

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Summary.—Pig dermal collagen was prepared by treating whole skin with a solution of crystalline trypsin at temperatures below 20°. The purified dermal collagen was implanted subcutaneously and into full thickness excised skin wounds in the pig. Biopsy specimens were removed after various periods of time and examined histologically. A parallel series of control experiments involving auto- and homografted skin were carried out under the same conditions, together with observations on full thickness excised wounds. Autografts and homografts behaved as previously described, the autografts appearing normal after 35 days while homografts were uniformly rejected and sloughed by Day 20 and contractions occurred as in open wounds. The dermal collagen grafts were invaded and colonized by host fibroblasts and other cells and became at least in part revascularized and re-epithelialized. The implanted collagen was progressively lysed and replaced by granulation tissue. Remnants of collagen persisted in peripheral parts of the graft up to Day 35 but had disappeared by Day 50. Dermal collagen implants covered with split thickness auto- and homografts suffered the same fate as the dermal collagen grafts. Dermal collagen implanted subcutaneously into adipose tissue achieved in some instances a state of permanence, the collagen bundles retaining their original form and containing fibroblasts and capillaries.

THE reaction which results in the destruction and sloughing of homologous skin grafts essentially derives from the production of antibodies to foreign antigens present in the grafted skin. Only in autografts, where such foreign antigenic material is absent, does the graft survive and continue to function normally. In the past, efforts have been made to block or desensitize the immune response (Medawar, 1946*a*, *b*; 1948). However, with the exception of immune tolerance induced in the embryo (Billingham, Brent and Medawar, 1956), this work has had but limited success.

An attempt to reduce homograft antigenicity by enzyme treatment was made by Dukes and Blocker (1952). These workers considered that nuclear material (nucleoprotein) was responsible for the stimulation of antibody production and they treated skin homografts with a deoxyribonuclease preparation by local application to the homograft at the time of grafting. They found that grafts treated with streptokinase–streptodornase persisted 62% longer than did similar grafts untreated with enzymes. They also concluded that the immune response

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was due mainly to antigen–antibody reactions taking place in the vessels supplying nutrients to the homologous tissue.

The principal objective of the present work was to provide a physiological substitute for skin homografts which did not stimulate an immune response. The bulk of the material responsible for the formation of antibodies to homologous connective tissue would appear to be cellular, together with noncollagenous intercellular and interfibrillar material (Sulitzeanu, 1965). Mature collagen is insoluble and has low or absent antigenicity.

It has been found that injection of rabbit antibodies to soluble collagen does not result in collagen damage in the rat (Rothbard and Watson, 1956). In view of the constancy of chemical composition of collagen, it was considered unlikely that mature collagen transplanted between individuals of the same species would result in antibody formation.

All cellular and noncollagenous material (apart from a few elastin fibres) can be removed from whole skin by treating it with a solution of crystalline trypsin at a temperature not exceeding 20°. Such purified dermal collagen has been implanted subcutaneously and into full thickness excised skin wounds in the pig and examined over varying periods of time. At the same time a series of control experiments involving auto- and homografted skin were carried out under the same conditions, together with observations on full thickness excised wounds.

MATERIALS AND METHODS

Eleven young large white pigs, 8 castrated males and 3 females, aged 8–12 weeks and weighing approximately 20 kg at operation were used. Donors of homograft dermal collagen and untreated homograft skin were aged between 2 and 4 months.

Anaesthesia was induced and maintained with halothane ("Fluothane") and oxygen. All operations were performed on the shoulder and anterior back region lateral to the spine which was gently washed with cetrimide ("Cetavlon"), shaved and then swabbed with 1% chlorhexidine ("Hibitane"). The measured graft donor or recipient sites were removed as full thickness skin. Such sites, which had a bed of subdermal fat, also provided full thickness loss open wounds.

Preparation of skin and dermal collagen grafts and implants.—After removal the skin was carefully trimmed to provide a thick split thickness of approximately 1 mm. Untreated autograft skin was placed in "199" culture medium until grafted. To prepare dermal collagen for grafts and implants the trimmed autograft or homograft skin was immersed in a solution of crystalline trypsin (Worthington Biochemicals), at a concentration of 2 mg/ml in 0·1 mol/l phosphate buffer solution, pH 9·0, with 0·5 mg/ml sodium azide as a bactericide, for 48 hours at room temperature (approximately 20°). The treated skin was then washed in saline, the epidermis wiped off and the hair shafts plucked. The skin was reimmersed in trypsin solution and, before grafting, was washed in 4 changes of normal saline and then bathed in "199" medium. Skin treated in this manner was effectively reduced to its dermal collagen component and appeared entirely free of cellular elements when examined histologically (Fig. 8).

Grafting procedure.—All grafts, which were either 1 × 1 in rectangles or 4 × ½ in strips, were positioned as fitting grafts onto the subdermal fat of the recipient site with minimal suturing. The grafts and open wounds were dressed with "Vaseline" gauze, on to which was placed folded swabs and then a block of foam rubber. The foam rubber was compressed with "Elasticon" tape bandaged around the animal's chest. Dermal collagen implants into granulation tissue or under split thickness grafts were similarly dressed, while subcutaneous implants were sited within subdermal fat beneath a reflected skin flap which was sutured back into position.

Experimental procedures included the following:

	Dermal collagen grafts		Dermal collagen implants (all homografts)	Autografts	Homografts	Open wounds
	Autografts	Homografts				
Number of grafts or implants	4	14	12	4	3	8
Number of biopsies	9	25	18	11	8	8

The grafts and open wounds were biosied between 5 and 50 days after operation and implants between 4 and 60 days.

Four of the 12 collagen implants were implanted as 1½-in long strips subcutaneously. Of the remainder, 4 1-in long strips were placed on subdermal fat onto which was grafted thin split thickness autograft skin and 2 further strips were similarly covered with thin split thickness homograft skin. Two strips of dermal collagen were also implanted into the granulation tissue of 10-day open wounds.

In addition, 12 dermal collagen grafts were treated with glutaraldehyde. Four of these grafts were immersed in cold (4°) 5% glutaraldehyde overnight before washing and grafting and, similarly, 2 grafts were treated with each of the following concentrations of glutaraldehyde: 0·01, 0·05 and 0·5%.

Grafts and open wounds were examined after 3–5 days, when they were first biopsied and dressings were generally removed after about 10 days. At biopsy, a thin slice cut deep into the subdermal fat was removed, which included the adjacent recipient skin as well as the graft or wound.

Histology.—All biopsy specimens were flattened onto strips of card and fixed in 10% formol saline, wax embedded and sectioned at 7 μ. The following stains were used routinely: haematoxylin and eosin, Masson's trichrome, Herovici's picropolychrome, Weigert's elastin stain and van Gieson's connective tissue method.

RESULTS

Untreated autografts, homografts and open wounds will be considered first, paying particular attention to dermal responses, as they act as controls for the dermal collagen grafts.

Autografts

Autografts behaved essentially as described by Hinshaw and Miller (1965), who studied the healing of skin grafts in the pig. At 5 days the graft epidermis had necrosed and was being undergrown by epidermis migrating from the surrounding recipient skin. Subepidermal oedema and the beginnings of collagenolysis were already apparent (Fig. 1). Thereafter, the new epidermis became hyperplastic and progressive dissolution of collagen throughout the graft was indicated by its paler staining properties and the presence of thinner collagen bundles and fibres (Fig. 2). Interbundle oedema and increased cellularity, caused mainly by capillary proliferation, were associated with this loss of collagen (Fig. 3). Remodelling of the edge and base of the graft collagen was occurring by Day 10 as the graft dermis was knitted into the surrounding scar tissue. On Days 35 and 50 the dermis appeared relatively normal and the graft, which showed no signs of contraction, was distinguishable from the surrounding skin only by its paucity of hair follicles and the thin band of ensheathing scar tissue. An interesting observation was the presence of several areas of degenerating and basophilic collagen which contained multinuclear giant cells in a graft at Day 10. These were identical to those appearing in collagen grafts (Fig. 12 and 13).

Homografts

By Day 5 the typical homograft rejection response was under way. The graft epidermis was dead and there was little or no overgrowth by the surrounding skin epidermis (Fig. 4). Intragraft capillaries were leaking erythrocytes, as occurred in some autografts, and mononuclears were present within the graft dermis, especially in association with the epidermal appendages. The developing granulation tissue beneath the graft was already thicker than that present under 5-day autografts. At Days 9 (Fig. 5) and 10 the greater thickness of the superficial graft dermis had necrosed and epidermis from the adjacent skin was migrating into the graft dermis at this level, overgrowing, at the graft margins, a strip of graft dermis. The base of the graft dermis was degenerating and being invaded by mononuclear cells which enclosed remnants of graft collagen fibres (Fig. 6). Although most of the graft had been sloughed, or lysed, and replaced by granulation tissue by Day 20, remnants of peripheral graft collagen were sometimes present within the scar tissue of the contracting wound (Fig. 7). At Days 35 and 50 no traces of the graft collagen were discernible and contraction had progressed as in open wounds.

Open wounds

At Day 5 the adjacent epidermis had migrated to the base of the wound and granulation tissue was developing diffusely within the upper region of the fat bed. By Day 10 granulation tissue now filled the original defect and was partially covered by migrating epidermis. Wound contraction had commenced and the completely epithelialized wound at Day 20 was further contracted and fine collagen fibres were present within the scar tissue. Thereafter contraction continued.

Dermal collagen grafts

Since autograft and homograft dermal collagen (Fig. 8) showed identical responses, they will be considered together. At Day 5 and after, the superficial quarter to third of the graft, which was filled with blood, had dried and necrosed and was separated by a polymorphonuclear leucocyte band from the underlying graft (Fig. 9 and 12). Epidermis from the adjacent recipient skin crossed the graft junction and then proceeded to migrate beneath this polymorph band (Fig. 11 and 13). Even by Day 5 the collagen beneath the polymorph layer was repopulated with capillaries and fibroblasts which were distributed between the collagen bundles. Lysis of the graft collagen, which started at this time, was associated either with an overt infiltration of granulation tissue or as a more diffuse process (Fig. 10). In some grafts at Day 5, and becoming a more general feature later, there arose small areas of basophilic collagen. These originated as small patches in the collagen bundles which then proceeded to expand and fuse (Fig. 12).

At Day 10 and thereafter the grafts generally showed a continuation of the above processes, with sloughing of the dried eschar, further migration of the epidermis and the progressive replacement of collagen by granulation tissue. There were now larger areas of basophilic collagen which were undergoing lysis and removal associated with the presence of multinuclear giant cells (Fig. 13). In all grafts there was good preservation of the peripheral zone, which had been overgrown by epidermis and was in fibrous communion with the host dermis

(Fig. 14 and 15). This graft remnant was not being lysed to the same extent by granulation tissue either from the graft bed or graft/skin junction. Apart from this peripheral region, by Day 20 granulation tissue had in most instances completely replaced the original graft collagen and the wounds were contracting exactly as if they were open wounds or had received untreated homografts. At Day 35 some wounds contained remnants of peripheral graft collagen but by Day 50 they were no longer detectable.

EXPLANATION OF PLATES

FIG. 1.—Autograft. 5 days. Necrotic graft epidermis being undergrown by epidermis from junction at left. Subepidermal oedema over graft and the beginnings of collagenolysis. Picropolychrome. × 24.

FIG. 2.—Autograft (junction at left). 9 days. Graft epidermis hyperplastic; more extensive subepidermal collagen loss. Graft dermis paler staining and has thinner collagen bundles and fibres. Masson's trichrome. × 22.

FIG. 3.—Autograft. Day 20. Hyperplastic epidermis; collagenolysis apparent throughout dermis associated with increased cellularity and small collagen fibres. Picropolychrome. × 24.

FIG. 4.—Homograft. Day 5. Necrotic graft epidermis and upper region of graft dermis dying. Junctional epidermis growing down edge of graft. Masson's trichrome. × 24.

FIG. 5.—Homograft. Day 9. Upper region of graft necrosed and filled with polymorphs. Mononuclears present in graft junction and throughout degenerating graft dermis. Junctional epidermis overgrowing edge of graft dermis. Masson's trichrome. × 22.

FIG. 6.—Homograft. Day 10. Predominantly mononuclear presence at base of graft which is being invaded and lysed. Picropolychrome. × 24.

FIG. 7.—Homograft. Day 20. Persisting remnant of edge of graft dermis (centre). Rest of graft has degenerated and sloughed and is replaced by scar tissue. Picropolychrome. × 24.

FIG. 8.—Cell-free, dermal collagen after trypsin treatment of skin. Picropolychrome. × 24.

FIG. 9.—Collagen homograft. Day 5. Upper region of graft necrosing (left) with epidermal overgrowth from the right. Haematoxylin and eosin. × 22.

FIG. 10.—Collagen homograft. Day 9. Lower region of graft highly cellular and undergoing diffuse collagenolysis. Note absence of immune response (compare Fig. 6). Masson's trichome. × 22

FIG. 11.—Collagen autograft. Day 10. Upper third of graft necrosed and being undergrown by junctional epidermis. Picropolychrome. × 24.

FIG. 12.—Collagen homograft. Day 10. A polymorph band separates dead collagen above from an area of more normal looking collagen. Lower third undergoing collagenolysis, with the development of basophilic areas and phaging multinuclear giant cells. Haematoxylin and eosin. × 90.

FIG. 13.—Collagen homograft. Day 11. Epidermis has grown beneath the upper necrotic collagen layer. An area of basophilic collagen has developed with multinuclear giant cells. While the collagen at left looks healthy it has been replaced with granulation tissue at right. Haematoxylin and eosin. × 75.

FIG. 14.—Collagen autograft. Day 20. Persisting region of graft collagen (right) with its smaller fibres knitted into recipient dermis. Picropolychrome. × 32.

FIG. 15.—Collagen homograft. Day 20. Persisting remnant of graft collagen (centre) with occasional fibres in granulation tissue at left. Picropolychrome. × 24.

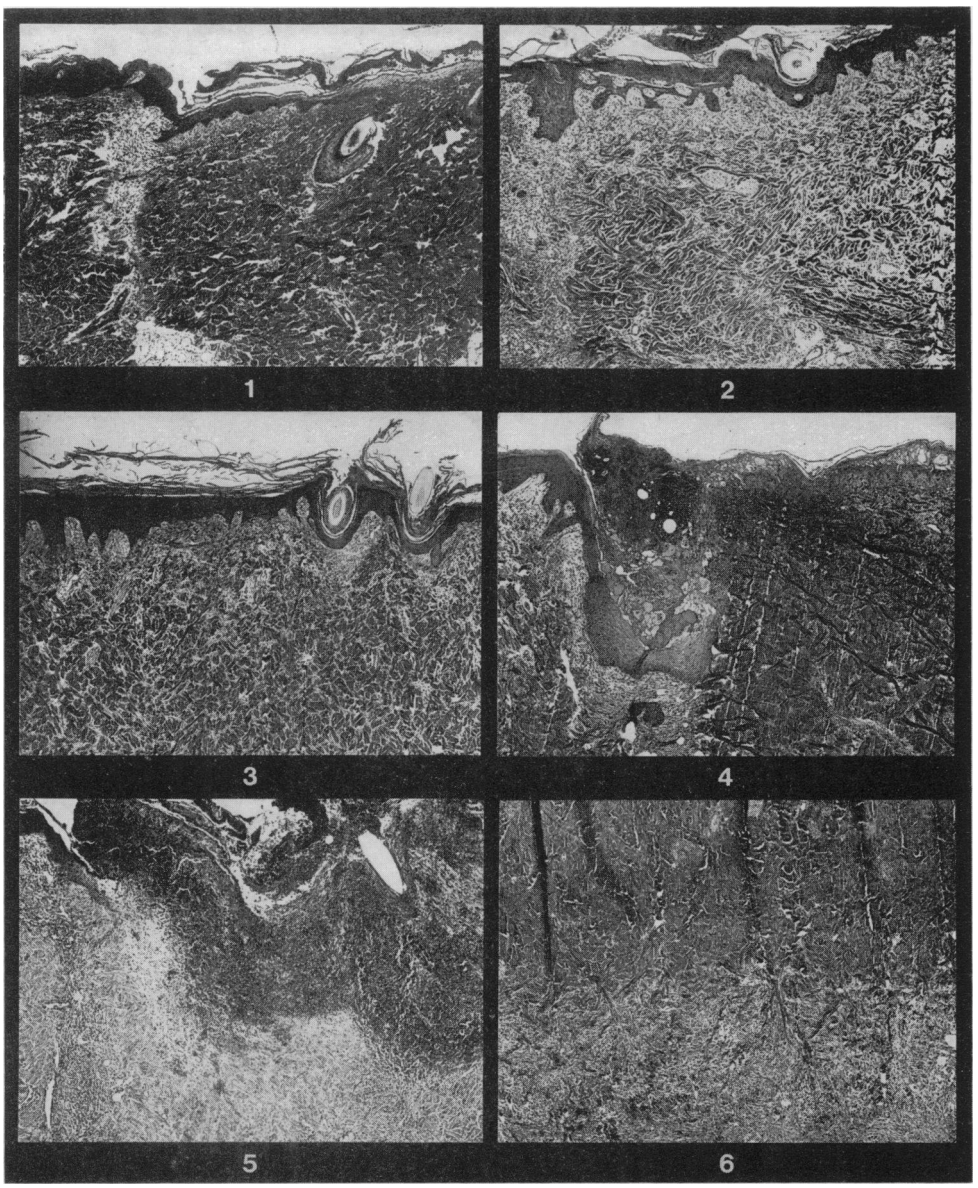
FIG. 16.—Collagen homograft with split-thickness autograft cover. Day 14. The dead split-thickness autograft overlies a layer of polymorphs then granulation tissue. The upper region of the collagen graft is undergoing lysis. Picropolychrome. × 22.

FIG. 17.—Collagen homograft with split-thickness autograft cover. Day 17. Further dissolution of graft collagen, whose remaining fibres are fine and becoming dispersed in the developing granulation tissue. Picropolychrome. × 24.

FIG. 18.—Subcutaneous collagen homograft. Day 9. Healthy looking graft with some peripheral collagenolysis. Masson's trichrome. × 24.

FIG. 19.—Subcutaneous collagen homograft. Day 20. Decrease in size of collagen bundles and fibres, especially in lower region. Picropolychrome. × 24.

FIG. 20.—Subcutaneous collagen homograft. Day 35. Persisting collagen graft ensheathed in thin band of scar tissue. Picropolychrome. × 24.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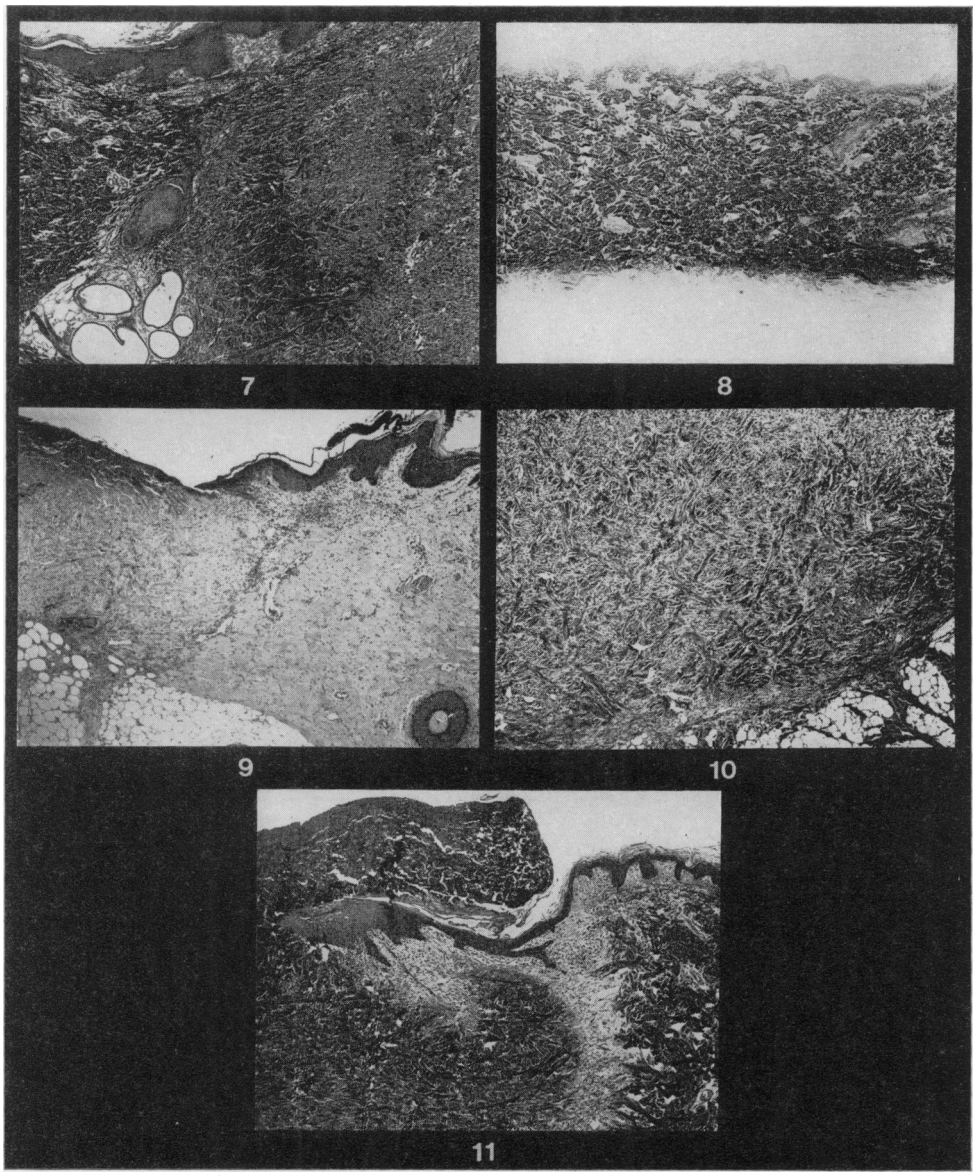

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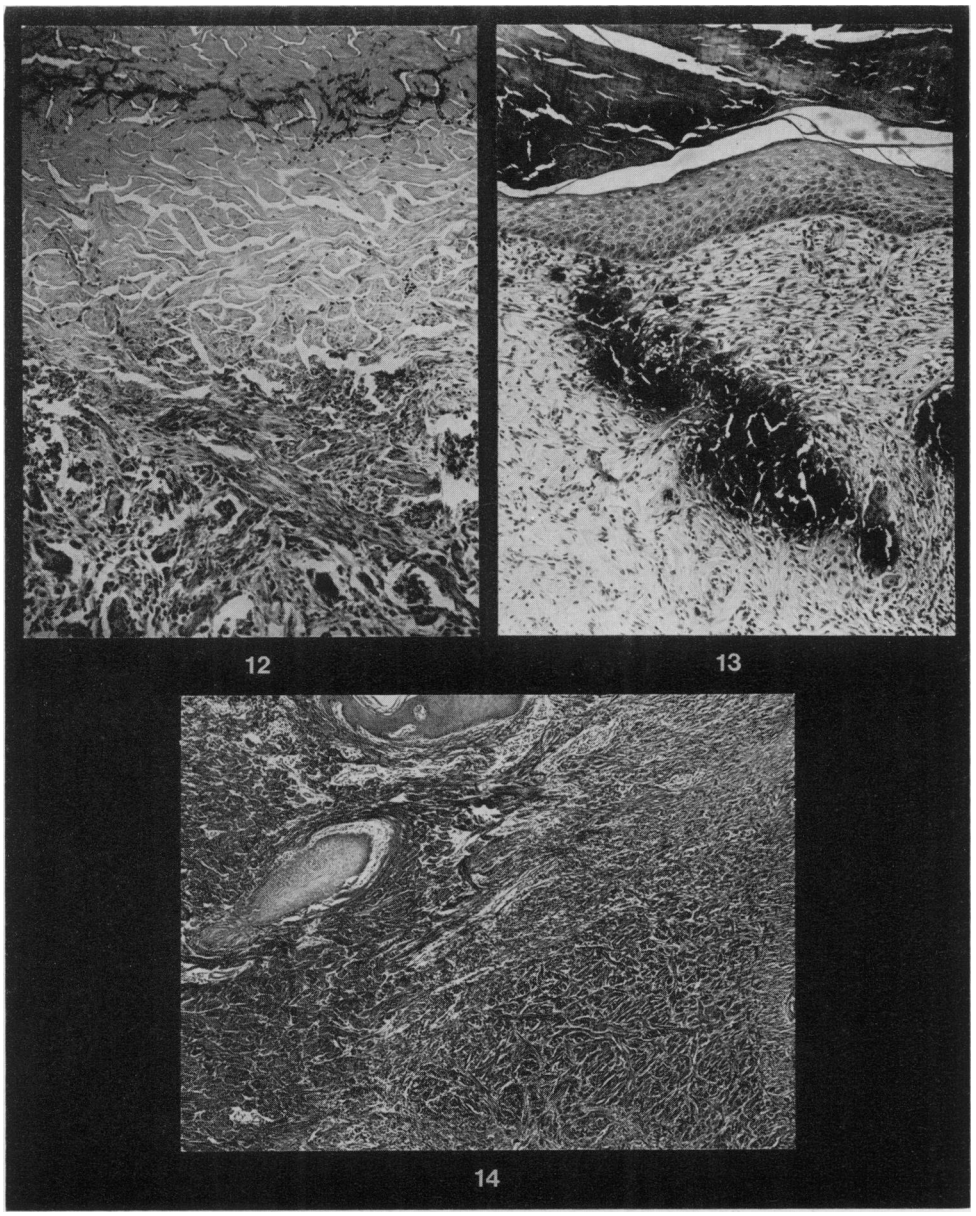

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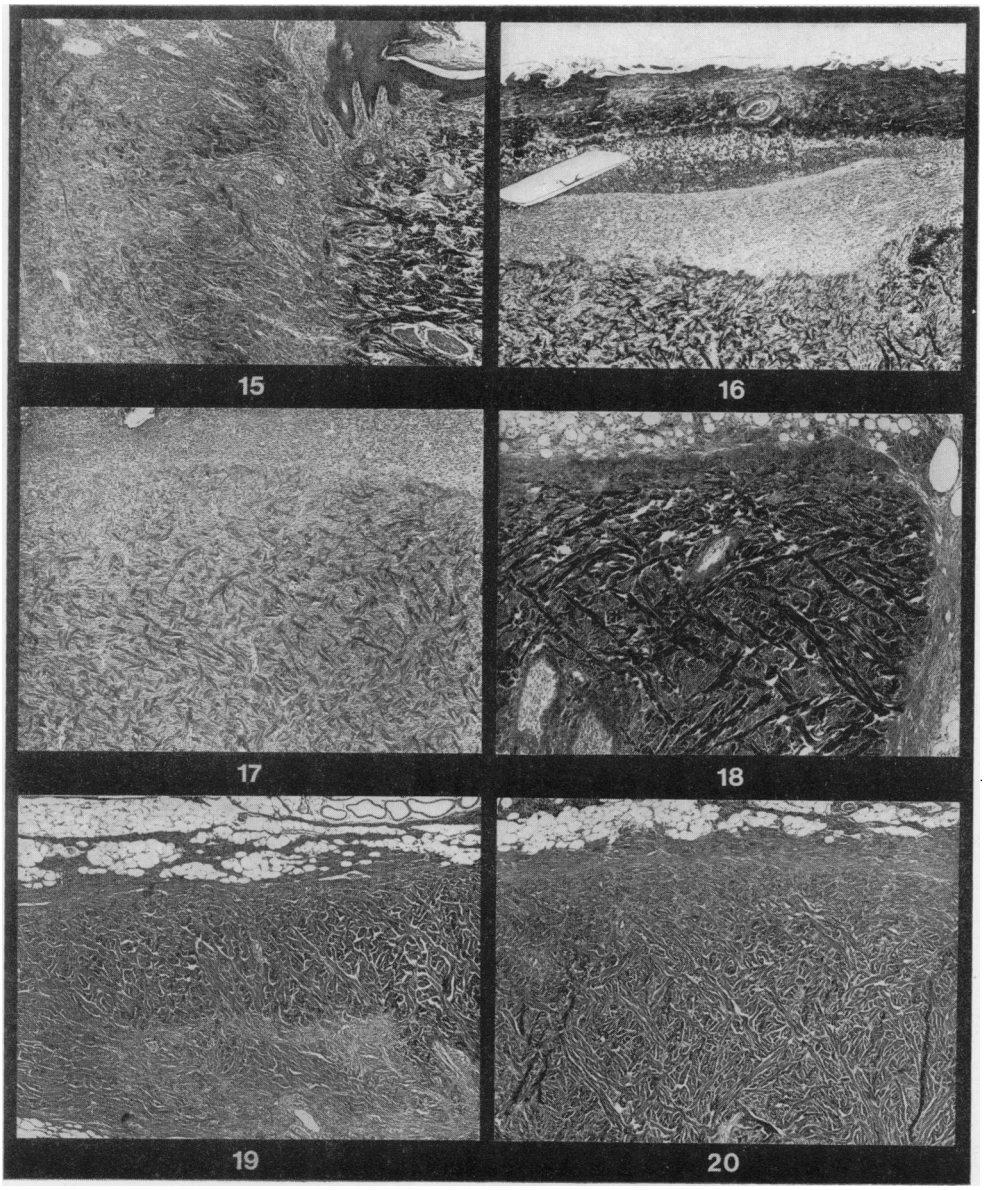
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Glutaraldehyde treated dermal collagen grafts

Dermal collagen was treated with glutaraldehyde in the hope that cross-linking would prevent or impede collagenolysis. While this was achieved, without exception all these grafts sloughed. Microscopic examination revealed that they had been undergrown by migrating epidermis and that no cellular infiltration of the grafts had occurred.

Dermal collagen implants

To prevent the loss of surface dermal collagen by drying, strips of dermal collagen were covered with split thickness autograft or homograft. This procedure slowed the process of dissolution of the collagen and its replacement by granulation tissue (Fig. 16 and 17), but they eventually suffered the same fate as grafts and by 20 days only remnants of collagen remained. While some of these remnants consisted only of a sparse fibrous network, there were instances of good bundle preservation.

Strips of dermal collagen implanted into 10-day granulation tissue also succumbed to gradual replacement by granulation tissue so that by 25 days the original implants were represented by a few widely spaced fibres which had disappeared by Day 50.

Dermal collagen implanted subcutaneously into adipose tissue showed the greatest resistance to lysis and replacement by granulation tissue (Fig. 18–20). Such implants, in varying degree, also suffered the appearance of basophilic regions with the presence of phaging multinuclear giant cells and some progressive replacement at the periphery by granulation tissue. However, this occurred more slowly than in the other types of implants and grafts and the extensive remnants which were present at Day 35, and which were ensheathed in scar tissue, had achieved an apparent state of permanence which was confirmed in biopsies at Day 60. These collagen remnants were composed of collagen bundles arranged in their original network and were populated by fibroblasts and contained capillaries.

DISCUSSION

Purification of dermal collagen

The digestion of collagen by trypsin is temperature dependent. At 37° collagen fibres exposed to trypsin at pH 8 show severe damage and partial digestion when examined with the electron microscope using a negative stain (Brenner and Horne, 1959). However, when treated at temperatures below 20° no damage to the fibres was obvious with the electron microscope; the temperature effect would appear to depend on thermal denaturation effects. Collagen heat denatured at temperatures above 50° is completely digested by trypsin. The non-helical (telopeptide) portion of the collagen molecule is also susceptible to enzymes other than collagenase. However, in most native collagens the lysine in the non-helical region is incorporated into a crosslink and hence trypsin does not attack this region. Trypsin only appears to split the non-helical region in salt-soluble lathyritic collagen (Bornstein, Kang and Piez, 1966; Kang and Gross, 1970). Examination of collagen fibres treated with a solution of crystalline trypsin as described in the present work showed no structural changes when examined either by light or electron microscopy (Cox, unpublished).

Fate of collagen

It was hoped that the purified dermal collagen implants would become colonized by fibroblasts and other cells from the host, which, together with a process of overgrowth of epidermis from surrounding skin, would lead to the establishment of an essentially normal skin structure. To some extent these objectives were achieved. The primary requisites for the success of these grafts were considered to be that the implanted dermal collagen fibres (1) should persist unaltered for a long period, thus providing an essential structural framework for the reformation of the vascular and the cellular elements of the tissue, (2) should not evoke foreign body reactions or immune responses, leading to the eventual destruction of the newly cellularized graft, (3) would provide a suitable dermal bed for the growth and development of normal epidermis and (4) would suppress the formation of granulation tissue.

Before discussing the fate of collagen grafts and implants, it is instructive to first consider the behaviour of auto- and homograft skin. Hinshaw and Miller (1965) made a detailed study of the healing of split- and full thickness skin autografts in the pig and considered that extensive collagen changes occurred in the grafts. They interpreted their histological findings as demonstrating almost total degradation of collagen, with its eventual restoration to nearly normal architecture. The present less extensive work supports this interpretation.

Untreated homograft skin behaved in the usual way and by Day 10 the grafts had generally necrosed and were in process of sloughing. As has been commonly observed, remnants of graft collagen persisted and were present at Day 20 in the scar tissue, but at Days 35 and 50 no graft collagen was discernible and the process of contraction and scarring occurred as in open wounds. This indicates that an active process of lysis of the graft collagen was taking place presumably through the agency of endogenous collagenase.

The dermal collagen grafts presented quite clear-cut differences in behaviour to either homo- or autografted whole skin. The deeper sited dermal collagen which did not dry out was quickly repopulated with capillaries and fibroblasts. However, with the passage of time a general process of replacement of dermal collagen with granulation tissue supervened although the peripheral part of the graft, which showed a fibrous union with the dermis collagen, persisted for the longest interval. Nevertheless, the general result was destruction and lysis of the implanted dermal collagen fibres, unaccompanied, however, by a typical homograft reaction. Collagenolysis presumably resulted from the localized liberation of collagenase which was associated with a foreign body type reaction and the development of granulation tissue. However, the main point to be noted was that cellular repopulation of the dermal fibres did occur, granulation tissue developed as an intragraft collagen replacement instead of arising as a primary underlying response and epidermis overgrew at least the peripheral parts of the graft, giving an approximately normal, though temporary, appearance to this part of the graft. A recurring feature of the collagen grafts, and implants, was the appearance of basophilic regions, possibly indicating calcification. This also occurred in an untreated autograft and may have resulted from damage to the grafts incurred in their preparation. In the case of the dermal collagen implanted under split thickness autografts or homografts or into 10-day granulation tissue, the process of dissolution of collagen fibres was retarded but not abolished although

some remanants of the original collagen persisted for a considerable time without much change. Again, destruction of the implanted collagen would appear to be due to the local liberation of collagenolytic enzymes.

In contrast to the high collagenase activity in the dermal grafts and implants described above, it is interesting to note the longer persistence of dermal collagen implanted into adipose tissue. Some of the collagen appeared to survive on a permanent basis, becoming ensheathed in scar tissue, repopulated by fibroblasts and supplied with nutrients by new capillaries. In this last instance we achieved partially the original objectives of this work *i.e.* to produce a permanently cell repopulated, vascularized and viable state in the transplanted purified dermal collagen, albeit in a different site. Here the grafted collagen seemed more nearly to respond as if it were untreated autograft skin. Collagenolysis occurred, but where collagen persisted there was retention of the original fibrous architecture although the bundles and fibres were decreased in size.

It has been shown that cultures of human skin, but not tissue extracts, liberate a collagenase which acts on collagen in the native fibrillar form and intact tissue collagen under physiological conditions (Eisen, Bauer and Jeffrey, 1970; Eisen, Jeffrey and Gross, 1968). Hence the release of this enzyme from both the epidermal and dermal component of skin during the healing phase following implantation could well account for the destruction and loss of the cutaneously grafted dermal collagen. However, collagenase synthesized at these sites would presumably have little or no effect on the collagen implanted in subdermal adipose tissue.

It would appear that another source of collagenase which leads to absorption of neighbouring collagen fibres is granulation tissue. This concept is supported by studies on the granulomatous reaction stimulated by subcutaneous injection of carrageenin (sulphated polygalactose) (Pérez-Tamayo, 1970). In this instance new connective tissue matures into adult collagen fibres, which subsequently break down and are reabsorbed. Here it is of particular interest that collagen is lost from the original overlying dermis (Williams, 1957). Despite partial remodelling in later stages, the dermis suffers a permanent and substantial loss of collagen. Such results seem to indicate the release of collagenolytic enzymes from the fibroblasts in the granuloma tissue and this phenomenon resembles the present experiments in which the liberation of collagenase from adjacent granulation tissue appeared to contribute to the loss of collagen. It should also be noted that collagen degradation resulting from local necrotic ulceration also occurs in distal insoluble collagen as well as that in the injured site (Houck *et al.*, 1962).

There is also evidence for a balance between synthesis and degradation of collagen in the body under normal conditions apart from localized collagenase activities in bone (Walker, 1966) and uterus (Grant 1965*a*; Gross and Lapiere, 1962). Chen and Postlethwait (1961) have shown that depletion of vitamin C (which is essential for collagen biosynthesis) results in a fall in tissue collagen. The dissolution of collagen occurs faster than its biosynthesis. Any factor tending to upset the natural balance in favour of lysis, such as the trauma associated with grafting as well as lack of vitamin C, might be expected to result in a fall in tissue collagen.

Bearing these factors in mind, an essential requirement would appear to be to provide a means of protecting the implanted dermal collagen against collagenase liberated by invading cells or adjacently situated healing tissues. It has been found that glutaraldehyde crosslinked collagen is completely resistant to bacterial

collagenase (Grant, 1965*b*). However, when such cross-linked collagen was grafted into excised wounds, it became undergrown by migrating epidermis and no cellular infiltration of the grafts occurred. This indicates that the host cells, and perhaps in particular fibroblasts, can recognize a chemically altered collagen even though the glutaraldehyde reaction involves only one of the amino acids in collagen (lysine). Hence, any method of inducing resistance to locally liberated collagenase should have a minimal effect on the chemical composition of the dermal collagen. One possible approach is by radiation induced crosslinking (Grant *et al.*, 1970) and it is hoped to extend the present work by the use of irradiated dermal collagen grafts.

Although the bulk of the grafted purified dermal collagen was subsequently broken down and reabsorbed, the use of such grafts may eventually prove to be of practical value. The fact that a typical homograft reaction was absent and also since it appears that it is the dermal component of a graft which inhibits the contraction of open wounds (Billingham and Reynolds, 1952; Gillman, Hathorn and Penn, 1956), sheets of purified dermal collagen might possibly be used as physiological dressings for large open wounds, together with a suitable covering. The advantage of using trypsin purified dermal collagen as opposed to collagen which has been dissolved in acetic acid or alkali and reconstituted (Abbenhaus *et al.*, 1965) is that at grafting the morphology and ultrastructure of the collagen fibres are preserved intact.

Furthermore, while much valuable information has been gained by the use of plastic sponge implants to study fibroblast infiltration and the formation and maturation of collagen, such experiments are basically unphysiological by virtue of the foreign element introduced, even though the plastic is chemically inert. The use of trypsin purified dermal and other collagen for implantation in the skin and other tissues may provide additional information and offers a simple system for studying the *in vivo* action of collagenase inhibitors to prolong the lives of the grafts.

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